Sperm quality and pregnancy rate after COX-2 inhibitor therapy of infertile males with abacterial leukocytospermia

Laura Gambera1,4, Francesca Serafini1,4, Giuseppe Morgante1,4, Riccardo Focarelli2, Vincenzo De Leo1,4 and Paola Piomboni3,4,5

1Department of Pediatrics, Obstetrics and Reproductive Medicine, 2Department of Evolutionary Biology, 3Department of Surgery, Biology Section, University of Siena, for the Diagnosis and Treatment of Couple Sterility, Siene, Italy and 4Center for the Diagnosis and Treatment of Couple Sterility, Azienda Ospedaliera Universitaria Senesente, Policlinico Le Scotte, Viale Bracci, Siena, Italy
5To whom correspondence should be addressed at: Department of Surgery, Biology Section, University of Siena, for the Diagnosis and Treatment of Couple Sterility, S. Maria alle Scotte Hospital, Viale Bracci, 53100 Siena, Italy. Tel: +39 0577 586632; Fax: +39 0577 586632; E-mail: piomboni@unisi.it

BACKGROUND: Leukocytes are a frequent finding in seminal plasma of infertile males with abacterial inflammation. We evaluated the effects of treatment with rofecoxib, a cyclooxygenase-2 inhibitor, on sperm quality and pregnancy rate after intrauterine insemination (IUI) or monitored intercourse. METHODS: We selected 47 infertile patients referred to our sterility centre for semen analysis. Sperm evaluation was performed by light microscopy with Papanicolaou and eosin staining, before and 1 month after therapy. Swim-up selection was carried out in two steps. Starting 6–8 weeks after the end of therapy, couples underwent different procedures of assisted fertilization according to their semen parameters. RESULTS: Semen analysis 30 days after the end of therapy showed a significant reduction in leukocyte concentrations with respect to baseline, an improvement of sperm motility and morphology, particularly the presence and shape of the acrosomal complex and tail structure. After monitored intercourse and IUI, pregnancy rate was 15.8 and 11.3%, respectively. CONCLUSIONS: Our results suggest that a decrease in leukocytospermia after rofecoxib therapy was associated with recovery of all seminal characteristics in basal and swim-up selected samples. This general improvement could justify the positive outcome of ART after anti-inflammatory therapy.

Key words: COX-2 inhibitor/infertility/leukocytospermia/pregnancy rate/sperm quality

Introduction

Leukocytes are a frequent finding in seminal plasma of infertile male patients, even in the absence of inflammatory symptoms. In many cases, leukocyte concentrations in seminal fluid are ≥1 × 10⁶ ml⁻¹, a pathological level according to the WHO (1999) criteria. Granulocytes are the most prevalent white blood cells (WBCs) in semen (50–60%), followed by macrophages (20–30%) and T-lymphocytes (2–5%). The frequency of leukocytospermia among infertile males is 30%, and in ~80% of leukocytospermic patients, no microbial infection can be detected in seminal fluid. The correlation between leukocytospermia and male infertility is controversial: some studies have failed to find any association (Fedder et al., 1999; Whittington et al., 1999; Sharma et al., 2001; Alvarez et al., 2002). Different studies suggest a negative effect of leukocytes and reactive-oxygen species, primarily produced by leukocytes, on semen quality (Sharma et al., 2001; Erenpreiss et al., 2002; Saleh et al., 2002).

There have been few reports of pharmacological treatment of leukocytospermia with anti-inflammatory drugs. Lackner et al. (2006) recently suggested that anti-inflammatory medication of 12 males with abacterial leukocytospermia improved sperm count and reduced leukocyte concentrations. Oliva and Multigner (2006) reported a significant increase in sperm motility and morphology after antihistamine-like drug treatment, with a significant decrease in leukocytes in semen.

© The Author 2007. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology.
All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org
Non-steroid anti-inflammatory drugs (NSAIDs) have been shown to inhibit the production of prostaglandins (PGEs) (Dawood et al., 1993), which are synthesized from arachidonic acid by the action of cyclooxygenase (COX) enzymes. The principal mechanism of NSAIDs is COX inhibition. COX exists in two isoforms: COX-1 and COX-2. The former is constitutively expressed and is involved in PGE synthesis in many tissues and organs, such as the testis. COX-2 is induced by inflammatory cytokines, growth factors and endotoxins, and the inhibition of COX-2 is responsible for the anti-inflammatory effects of NSAIDs (Bolten, 1998). A newer class of COX-2 inhibitor drugs was developed for use in patients with osteoarthritis and/or acute pain. These drugs selectively inhibit COX-2 activity in a dose-dependent manner, lowering PGE concentrations. There is no significant inhibition of COX-1 activity. NSAIDs have been shown to have a positive effect in the co-treatment of inflammatory urogenital tract pathology, but their effect on sperm quality and the outcome of assisted reproductive procedures has never been investigated.

In infertile males with reproductive difficulties related to abacterial inflammation of the urogenital tract, we evaluated the effects of a COX-2 inhibitor on sperm quality and pregnancy rate after intrauterine insemination (IUI) or monitored intercourse.

Materials and methods

Patients
Among patients referred to our sterility centre between September 2005 and May 2006 for semen analysis after 12–18 months of sexual intercourse without conception, we selected 47 males on the basis of leukocyte count (>1 000 000 ml⁻¹) in seminal plasma frequently associated with increased ejaculation volume (~6 ml⁻¹). Azospermic patients were excluded from the study. In order to confirm the diagnosis of leukocytospermia, two semen analyses were performed at 1 month interval and the last one was used for statistical analysis. Medical history, physical examination and serum concentrations of hormones [total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone, estradiol (E₂), and β-inhibin] were evaluated. In all patients, microbiological analysis of seminal fluid was performed at the Unit of Microbiology, Siena Hospital, in order to detect common bacteria, such as *Mycoplasma, Trichomonas vaginalis* and *Chlamydia trachomatis*. Bacterial cultures were considered positive if the number of colony-forming units of pathogenic bacteria resulted ≥1 × 10⁵ ml⁻¹.

The female partners underwent complete infertility workup, including hysterosalpingography. No health or fertility problems were found. None of the women had untreated hormonal irregularities.

Pharmacological treatment
Selected patients without seminal-fluid bacterial infection associated with leukocytospermia were treated with a daily dose of 25 mg of rofecoxib in capsules, for an uninterrupted period of 30 days. WHO-recommended methods (1999), using an inverted polarized Olympus IX521 microscope, equipped with Hoffmann lens and plate at 37°C. Eosin Y staining was used to detect necrotic sperm.

In each step of therapy, morphological analysis of 100 sperm per sample was performed using the Papanicolau staining method (Oettle, 1986). Morphological characteristics of sperm organelles (nucleus, acrosomal and postacrosomal region and tail) were evaluated according to the WHO (1999) criteria. We compared statistically the two steps: at the beginning and the end of therapy.

Peroxidase staining
Leukocytes were counted by the method of Politch et al. (1993) adapted by Endtz (1974). Briefly, 0.0375% H₂O₂ was added to 4-ml benzidine stock solution (0.025% v/v benzidine, Sigma Aldricht, in 50% ethanol). Twenty microlitres of ejaculate was mixed with 20 µl fresh benzidine–H₂O₂ solution. After 5 min of incubation, 160 µl of PBS was added and peroxidase-positive (round, brown stained) and peroxidase-negative (unstained) cells were counted in a Mackler chamber using a phase-contrast microscope.

Swim-up procedure
Swim-up selection was performed before and after therapy to compare sperm recovery in the two steps. Aliquots (1 ml) of samples were gently mixed with an equal volume of sperm medium (COOK, Italy) and centrifuged at 300 g for 10 min. The supernatant was discarded and the pellet layered with 600–1000 µl fresh sperm buffer (COOK) and incubated for 40 min at 37°C in tubes inclined at an angle of 45°. Finally, 0.3–0.5 ml of the upper layer was aspirated and analysed.

Statistical analysis
The data were collected and analysed by means of the commercial software GraphPad Prism4 (GraphPad Software Inc., San Diego, CA). Results were expressed as mean ± SD. The signed rank test was used for comparisons. Statistical significance was set at *P* < 0.05.
The number of leukocytes decreased from a mean of 5.6 ± 2.1 ml to 3.6 ± 1.1 ml. Eosin staining was performed to detect common bacteria, such as Micoplasma, Trichomonas vaginalis and Chlamydia trachomatis, and we registered in 7.8% of sperm showed malformed nucleus; rolled-up sperm tail was detected in 63.9% of observed cells.

In order to evaluate the fertility potential of semen samples, swim-up selection was performed before therapy. When swim-up selection was repeated 30 days after the end of therapy, IUI was performed in 28 couples. Sperm concentration, progressive motility and morphology in swim-up selected samples used for IUI were comparable to the values observed after rofecoxib therapy. Leukocyte concentration was lower than 1 × 10^6 in all basal samples. A total of 62 IUI cycles were performed and 7 clinical pregnancies were obtained (pregnancy rate per cycle 11.3%). The other 19 couples with treated male partners performed monitored sexual intercourse and three clinical pregnancies (15.8%) were obtained (Table II).

**Discussion**

In evaluating the effects of anti-inflammatory treatment on sperm parameters of infertile men with abacterial inflammation of the urogenital tract, we found a slight positive effect. The effects of NSAIDS on inflammatory seminal fluid pathology are not well documented. In a recent study, Lackner et al. (2006)

### Table I. Sperm parameters evaluated before and after therapy in basal and swim-up-selected samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal (n = 47)</th>
<th>After Therapy (n = 47)</th>
<th>Swim-up (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (10^6 ml^-1)</td>
<td>25.1 ± 7.6</td>
<td>29 ± 9.9</td>
<td>8.2 ± 4.1</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>23.3 ± 10.1</td>
<td>46.9 ± 17.1*</td>
<td>48 ± 8.6</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>5.6 ± 2.1</td>
<td>3.6 ± 1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Leukocytes (10^6 ml)</td>
<td>1.6 ± 1.1</td>
<td>0.4 ± 0.2*</td>
<td>0</td>
</tr>
<tr>
<td>Normal sperm morphology (%)</td>
<td>19 ± 4.8</td>
<td>34.1 ± 7.3**</td>
<td>28 ± 6.2</td>
</tr>
<tr>
<td>Eosin staining (positive %)</td>
<td>54.7 ± 14.1</td>
<td>28.4 ± 8.9**</td>
<td>Not performed</td>
</tr>
</tbody>
</table>

*Signed rank test: P ≤ 0.05.
**Signed rank test: P ≤ 0.01.

### Table II. Pregnancy rate after intrauterine insemination (IUI) or monitored intercourse starting 6–8 weeks after the end of therapy of male partner

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>47</td>
</tr>
<tr>
<td>Male age (years)</td>
<td>29–41</td>
</tr>
<tr>
<td>Female age (years)</td>
<td>27–35</td>
</tr>
<tr>
<td>Number of couples treated with IUI</td>
<td>28</td>
</tr>
<tr>
<td>Number of cycles</td>
<td>62</td>
</tr>
<tr>
<td>Pregnancy rate per IUI cycle (%)</td>
<td>11.3</td>
</tr>
<tr>
<td>Number of couples performing intercourse</td>
<td>19</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>15.8</td>
</tr>
</tbody>
</table>
demonstrated a positive effect of an anti-COX-2 drug on seminal parameters of a small number of patients after only 2 weeks of therapy.

Our study suggests that the COX-2 inhibitor has positive effects on sperm parameters of patients with asthenoteratozoospermia associated with leukocytospermia and, frequently, with increased ejaculate volume. These characteristics of seminal fluid could be responsible for the long duration of infertility. Inflammation of the urogenital tract, associated or otherwise with microbial infection, is often a cause of reduced fertility, although there is no consensus about whether and how male genital-tract inflammation affects sperm fertilizing potential. Leukocyte concentration is normally $< 10^6$ ml$^{-1}$, but it increases in inflammatory conditions. As granulocytes are the main constituent of WBC in semen, Reinhardt et al. (1997) demonstrated that granulocyte elastase, determined by an enzyme immunoassay, is a very specific marker of male genital inflammation and Zorn et al. (2004) correlated the presence of seminal elastase--inhibitor complex, a marker of urogenital inflammation, with negative outcome of in vitro fertilization (IVF), in patients with asymptomatic inflammation. The influence of leukocytes on seminal fluid is controversial, despite its high incidence among infertile men, ranging from 15 to 28%, whereas in fertile men it is 10% (Zorn et al., 2000). Few studies failed to detect a negative effect of leukocytes on sperm quality: Tomlinson et al. (1992) even suggested that they have a role in the removal of abnormal spermatozoa from the ejaculate, increasing the percentage of sperm with normal morphology, and Kaleli et al. (2000) have not observed sperm damage during leukocytospermia. On the contrary, many other authors have found evidence that WBCs are significant cofactors of male infertility, influencing sperm number, motility and functions in a negative way (Wolff et al., 1990; Yanushpolsky et al., 1996; Thomas et al., 1997; Aziz et al., 2004), as also demonstrated by the hamster-ovum penetration test, an important prognostic factor for IVF (Wolff, 1995).

Because of the absence of clinical symptoms, the origin of the leukocytes is unclear. Normally, most leukocytes appear to originate from the epididymis because vasectomized men show very few leukocytes in semen. Sperm damage by WBC can also be mediated by reactive-oxygen species, proteases and cytokines. Moreover, Erenpreiss et al. (2002) suggested that leukocytes induce DNA damage in a cascade-like manner, particularly in sperm with poor morphology and motility. DNA fragmentation also affects the outcome of assisted reproductive technologies.

Our results clearly indicated a decrease in leukocytospermia associated with recovery of all seminal parameters including motility and morphology in basal and swim-up selected samples. This general improvement could justify the positive outcome of assisted reproduction procedures carried out after anti-inflammatory therapy. The resulting percentage of clinical pregnancy in our treated couples did not differ from that reported for the general population of infertile couples. Nevertheless anti-inflammatory treatment improved semen quality, allowing these couples to become eligible for less invasive treatment of assisted reproduction, as monitored intercourse or IUI. Inflammation reduces evidently the chance of pregnancy by impairing the fertilizing potential of sperm.

References


Submitted on August 22, 2006; resubmitted on November 14, 2006; accepted on November 29, 2006